# REVERSIBLE ACTIVATION AND INCOMPLETE MEMBRANE FORMATION OF THE UNFERTILIZED EGGS OF THE SEA URCHIN.[1]

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1. The writer showed in 1913 that the artificial activation of the egg can be reversed under certain conditions.[2] When the egg of *Arbacia* is treated with a base in the proper concentration and for the proper length of time the effect is somewhat similar to that which follows the treatment of the egg with a fatty acid. The egg is induced to develop, it may segment (as a rule abnormally) once or twice, and if nothing else happens it will perish rather rapidly (unless the developmental processes are prevented). If the eggs after the treatment with the acid or with the base receive a treatment with a hypertonic solution of the proper concentration and for the proper time they may develop into larvæ.

The writer found that if the eggs are treated with alkali in the proper way to induce development and if they are immediately afterwards put for several hours into a solution which prevents their development (sea-water with chloral hydrate or NaCN) the eggs when taken out behave as if nothing had been done to them. They neither segment nor do they disintegrate and they can again be induced to develop by fertilization (or probably with the methods of artificial parthenogenesis though the writer has not yet tried this). The activating effect of the alkali is therefore reversible.

This reversibility is, however, only possible if the eggs have not been exposed to the alkali too long. After too long an exposure the effect of the alkali is no longer reversible by a temporary suppression of the developmental processes. A second condition for the reversibility is that the eggs are immediately transferred from the alkaline solution into the chloral hydrate

[1] From the Rockefeller Institute for Medical Research, New York.

[2] Loeb, *Science*, N. S., XXXVIII., 749, 1913; *Arch. f. Entwcklngsmech.*, XXXVIII., 277, 1914.

or NaCN solution. If they are first transferred into normal sea-water and then after fifteen or thirty minutes later are transferred to the cyanide solution nothing may happen to them as long as they remain in the cyanide solution (unless the HCN evaporates) but they will disintegrate when put back into normal sea-water. From this we must conclude that in the sea-water the processes started in the alkaline solution will continue and the egg behaves as if it had been overexposed to the hyperalkaline solution.

Somewhat similar experiments were made with the eggs of *Arbacia* which had been treated with butyric acid. When such eggs were put over night into the cyanide-sea-water immediately after the artificial membrane formation they did not as a rule disintegrate when put back into normal sea-water but could be fertilized afterwards. The writer is, however, not certain that the phenomenon of reversibility is as constant here as in the case of the alkali treatment.

The writer pointed out that the explanation for this phenomenon could probably be found if we compare the behavior of the eggs of *Arbacia* with that of eggs of *purpuratus* under similar conditions. If we cause the production of a butyric acid membrane in the egg of *purpuratus* the activation of the egg is usually irreversible. In the egg of *purpuratus* the membrane which is formed under the butyric acid treatment is very tough and is separated by a wide area from the protoplasm, while in the egg of *Arbacia* the membrane consists often only of a fine gelatinous film which lies tightly around the egg. The writer concluded from this that there may be a quantitative if not a qualitative difference in the amount of change produced by the butyric acid treatment in the cortical layer of the two kinds of eggs; in the egg of *Arbacia* where this change is quantitatively smaller the activation is reversible, while in the egg of *purpuratus* where the change is quantitatively larger (and possibly also qualitatively different) it is irreversible (Loeb, "Artificial Parthenogenesis and Fertilization," Chicago, 1913, p. 286).

2. This idea is further supported by the curious phenomena of reversibility in the eggs of *Strongylocentrotus purpuratus*. When one tries to induce artificial parthenogenesis in the eggs

of *purpuratus* by a treatment with a hypertonic solution (without artificial membrane formation) it is found that this is possible only with the eggs of certain females. Such eggs develop into plutei. In the eggs of other females a small percentage of eggs will begin to segment and they may go to the 2, 4, 8, or 16-cell stage or still a little further but then stop developing. Such eggs will go into the resting stage again and are normal to all external appearances. If fertilized with sperm a number of hours or a day later the individual blastomeres will each form a special fertilization membrane and develop into small but apparently perfect larvæ.[1] This experiment shows that the activating effect which the hypertonic solution had was reversed, since all these eggs were at first in the state of active development.[2]

3. The writer wishes to report in this paper the fact that in the egg of *purpuratus* under definite conditions a membrane formation can be produced by butyric acid which leads neither to any development if followed by the usual treatment with a hypertonic solution nor to a rapid disintegration when not followed by any "corrective" treatment. Since in all previous observations on the effects of membrane formation by butyric acid the reverse was found it seemed of theoretical importance to study this exception.

The main condition for the experiment is that the eggs are put after the treatment with butyric acid into a $m/2$ solution of $NaCl + KCl + CaCl_2$ instead of into sea-water; the various salts are always used in the proportion in which they are contained in sea-water. The eggs were first washed three times in a mixture of $m/2$ $NaCl + KCl + CaCl_2$ and were then put for from one and a half to two and a half minutes into a mixture of 50 c.c. $m/2$ $NaCl + KCl + CaCl_2$ + 1 c.c. $N/10$ butyric acid; from here they were transferred into a neutral or a slightly alkaline solution of $m/2$ $NaCl + KCl + CaCl_2$. In this solution all

[1] Loeb, *Arch. f. Entwcklngsmech.*, XXIII., 479, 1907; "Artificial Parthenogenesis and Fertilization," Chicago, 1913, p. 237.

[2] Only the activating effect of the hypertonic solution is reversible, the second, corrective effect, which the hypertonic solution imparts to the egg was not reversed since these blastomeres develop also if only the artificial membrane formation is induced in them by butyric acid, while without the previous treatment with a hypertonic solution such eggs would soon perish. Loeb, *Jour. Exper. Zool.*, XV., 201, 1913; "Artificial Parthenogenesis and Fertilization," p. 238.

the eggs form a membrane which is very thin and which is at first at a great distance from the egg. Figs. 1 and 2 give an idea of the first appearance of this membrane; the membrane in Fig. 1 was formed in a neutral, that of Fig. 2 in a faintly alkaline solution. Later on, the membrane collapses and is found lying rather close to the egg. Such eggs can be readily fertilized with sperm in spite of the existence of the membrane. The latter is either naturally permeable for sperm or it is easily torn and thus allows the sperm to reach the egg or it is not entirely continuous around the eggs.

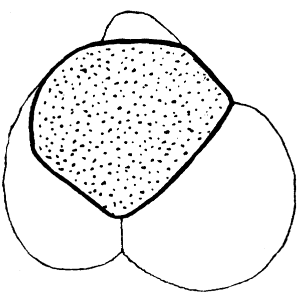

FIG. 1.

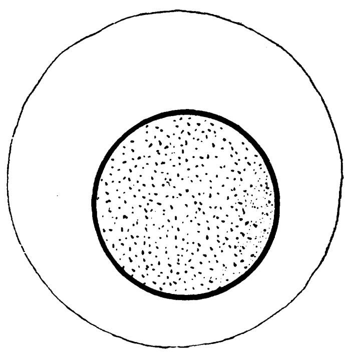

FIG. 2.

Eggs were washed three times in a mixture of *m*/2 NaCl + $CaCl_2$ + KCl and then put for from one and a half to two and a half minutes into 50 c.c. *m*/2 NaCl + KCl + $CaCl_2$ + 1.0 c.c. *N*/10 butyric acid. From this solution they were transferred:

(*a*) Into normal sea-water.[1]

(*b*) Into a neutral mixture of *m*/2 NaCl + KCl + $CaCl_2$.

Lot *a* formed normal fertilization membranes and disintegrated in a few hours. The eggs of lot *b* formed the fine fertilization membrane which was at first very distant from the egg. Some of these eggs were transferred into normal sea-water after one hour. These eggs did not form a better membrane but perished in the same way as the eggs of lot *a*.

A second portion of lot *b* was transferred into normal sea-water after having been for seven hours in the mixture of *m*/2

[1] Such eggs have at first a tendency to stick to the glass when transferred to sea-water. It is necessary to keep them in slight agitation for some time until they have lost their tendency to stick.

$NaCl + KCl + CaCl_2$. These eggs did not disintegrate during the following night although they did not appear quite normal the next day. The rest of the eggs remained in the $m/2$ $NaCl + KCl + CaCl_2$ solution for twenty-four hours. When these eggs were put into sea-water they did not disintegrate. To some of these eggs sperm was added the next morning and about 20 per cent. developed.

This experiment indicates that the nature of the membrane and the cortical changes which activate the egg of *purpuratus* are to a large extent determined by the solution into which the eggs are put after the butyric acid treatment. If the eggs are put directly into sea-water after the butyric acid treatment they form the typical membrane and rapidly perish if nothing else happens to them. The same may be the case if they are transferred from the butyric acid solution into a neutral solution of $m/2$ $NaCl + KCl + CaCl_2$ for a short time (less than one hour) and if they are afterwards transferred into normal sea-water. But if they remain in the $NaCl + KCl + CaCl_2$ solution for a number of hours they will as a rule not disintegrate if put back into sea-water.

A second experiment may be quoted. Eggs (that had been washed three times in a neutral $m/2$ $NaCl + KCl + CaCl_2$ solution) were put for one and a half to two and a half minutes at a temperature of about 14° into 50 c.c. of the same solution + 1 c.c. $N/10$ butyric acid. From here the majority of the eggs were transferred into a neutral solution of $m/2$ $NaCl + KCl$ $CaCl_2$ and the rest into sea-water. The latter formed normal fertilization membranes and disintegrated the same day. Those transferred into the neutral $NaCl + KCl + CaCl_2$ solution had all formed membranes which were at first separated from the egg by a great distance. They were kept in this solution over night and the next morning they were transferred into normal sea-water. All looked normal except that the membrane now formed a fine veil lying closely to the egg. Some of these eggs were fertilized with sperm. They all segmented and developed into swimming larvæ. The others were kept in sea-water without sperm to find out whether they would now disintegrate or whether the effect of the butyric acid treat-

ment had worn off. This was the case. The eggs no longer disintegrated in sea-water. A fraction of these eggs was fertilized later and segmented.

When the eggs are transferred into normal sea-water immediately after the butyric acid treatment they perish rapidly if no second treatment is given to them. If they are treated with hypertonic sea-water (50 c.c. sea-water + 8 c.c. 2 1/2 *m* NaCl or NaCl + KCl + $CaCl_2$) for from thirty to sixty minutes they will develop into larvæ. If they are treated longer they will begin to develop but form very abnormal larvæ.

If the eggs, however, after the butyric acid treatment are not put into sea-water but into a mixture of *m*/2 NaCl + KCl + $CaCl_2$ in which they form the thin membrane and if they are kept here until they no longer disintegrate in normal sea-water, they cannot be induced to develop when treated for from thirty to sixty minutes with a hypertonic solution. Such eggs have, therefore, in spite of their membrane formation gone back into the condition of a resting egg.

4. It was obvious from these experiments that the constituents of the sea-water determine the nature of the membrane after butyric acid treatment. The writer expected to find that Ca was an absolute prerequisite for the membrane formation but this was not the case, although the presence of $CaCl_2$ improves the character of the membrane. Eggs that had been taken out of a *m*/2 NaCl solution were treated with 50 c.c. *m*/2 NaCl + 1 c.c. *N*/10 butyric acid for one and a half to two and a half minutes. They were then transferred into the following three sets of solutions:

(1). NaCl + KCl,
(2). NaCl + $CaCl_2$,
(3). NaCl + KCl + $CaCl_2$,

which were made in duplicates, the one set remaining neutral, the second set faintly alkaline (0.5 c.c. *N*/100 NaOH were added to 100 c.c. of the solution). The result was not very different in the neutral and alkaline solutions. Many but not all the eggs put into NaCl + KCl formed very thin membranes often only in part of the egg. The membrane formation does therefore not require the addition of $CaCl_2$, but the eggs transferred

into $NaCl + CaCl_2$ or into $NaCl + KCl + CaCl_2$ had formed a better and apparently stronger membrane than those put into $NaCl + KCl$.

In another experiment it was found that in a mixture of $NaCl + MgCl_2$ in the proportions in which these two salts occur in the sea-water a membrane formation was also possible, but the membrane had the same fine and thin character. In all these cases the eggs could be fertilized with sperm in spite of the membranes. The writer is under the impression that no second membrane was formed upon the addition of sperm and only the hyaline membrane developed later. That the eggs after this inefficient membrane formation were not in the same condition as eggs which are put into normal sea-water after artificial membrane formation was shown by the fact that when put into a hypertonic solution for from thirty to sixty minutes not a single egg could be induced to segment.

The fact that the membrane always collapsed after some time seems to indicate that it soon became permeable for the colloids liquefied at the surface of the egg in the process of membrane formation.

5. The explanation of the phenomenon of ineffective membrane formation will probably be the same as that for the cases of reversible activation of the egg. It is an established fact that the rate of oxidations in the egg of *purpuratus* is raised about 600 per cent. by the act of fertilization. This rise is entirely due to the alteration of the surface layer of the egg which results in the membrane formation since artificial membrane formation alone has the same effect on oxidation. Even if the egg is killed in the process of membrane formation, *e. g.*, if we treat the unfertilized egg with saponin and leave it in this solution, the rate of oxidation is raised to the same amount.[1] The amount to which the artificial membrane formation raises the rate of oxidations in a sea-urchin egg seems to be a constant for each species and not to depend upon the nature of the agencies employed for this purpose.

When we treat the unfertilized eggs of *purpuratus* with a hypertonic solution (without inducing a membrane formation) the

[1] Loeb and Wasteneys, *Jour. Biol. Chem.*, XIV., 479, 1913.

eggs of some females will develop into larvæ, while the eggs of others will not. The writer assumed that the hypertonic solution if applied to the unfertilized egg has two effects, one consisting in the alteration of the surface layer (comparable to the effect of butyric acid) and secondly the corrective effect. Wasteneys and the writer[1] published a series of measurements on the influence of a hypertonic solution on the rate of oxidations in unfertilized eggs of *purpuratus*, and found that the effect varied enormously with the eggs of various females. The eggs were in the hypertonic solution for one hour and the rate of increase in oxidation varied for the eggs of various females between 40 and 400 per cent. As we stated above, the eggs of only a limited number of *purpuratus* can be induced to develop into larvæ by a mere treatment with a hypertonic solution and it is probable that the eggs in which the oxidations were raised efficiently were the ones that could be induced to develop into larvæ in this way, while those in which the rate of oxidations was not raised to the same height did not segment. We also noticed that in the latter type of eggs the rate of oxidations diminishes after some time. It is possible that the reversion of development in such cases is due to a decline in the rate of oxidations below the height required for development.

The same possibility holds for the lack of development of the eggs after artificial membrane formation through butyric acid when they are afterwards put into a solution of $NaCl + KCl + CaCl_2$ (instead of into sea-water). The fact that no correct membrane is formed and that the eggs neither develop nor disintegrate, and behave towards a treatment with the hypertonic solution like eggs without a membrane, arouses the suspicion that in this case the butyric acid treatment did not lead to the proper increase in the rate of oxidations. It is intended to investigate this possibility next summer, since it may also furnish the explanation of the phenomena of reversibility in development.

[1] Loeb and Wasteneys, *Jour. Biol. Chem.*, XIV., 474, 1913.